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Enterococcal Species Associated with Slovak Raw Goat Milk, Their Safety and Susceptibility to Lantibiotics and Durancin ED26E/7

Andrea Lauková *, **Valentína Focková** and **Monika Pogány Simonová**

Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovakia; fockova@saske.sk (V.F.); simonova@saske.sk (M.P.S.)
* Correspondence: laukova@saske.sk; Tel.: +421-557-922-964

Abstract: Goat milk has become a popular item of human consumption due to its originality. Enterococci are ubiquitous bacteria, and they can also be found in traditional dairy products. This study focuses on the safety of enterococci from Slovak raw goat milk and on their susceptibility to lantibiotic bacteriocins and durancin ED26E/7, which has not previously been studied. Biofilm formation ability in enterococci, virulence factor genes, enzyme production and antibiotic profile were investigated. Samples of raw goat milk (53) were collected from 283 goats in Slovakia. MALDI-TOF mass spectrometry identified three enterococcal species: *Enterococcus faecium*, *E. hirae* and *E. mundtii*, with dominant occurrence of the species *E. faecium*. Low-grade biofilm formation ability ($0.1 \leq A_{570} < 1.0$) was found in four strains of *E. faecium*. *Gelatinase*, *hyaluronidase*, *aggregation substance* and *enterococcal surface protein* genes were absent in these enterococci. Gene *efaAfm* (adhesin) was detected in five *E. faecium* strains. However, it was not detected in biofilm-forming strains. Enterococci detected in Slovak raw goat milk were found not to have pathogenic potential; four strains even produced high amounts of useful β-galactosidase. The strains were susceptible to lantibiotic bacteriocin treatment and to durancin ED26E/7 as well, which represents original information in dairy production.

Keywords: raw goat milk; enterococcal species; safety; virulence factor; bacteriocins

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1. Introduction

Enterococci are widespread microorganisms which can also be found in many traditional dairy products [1,2]. They belong in a community of lactic acid-producing bacteria which forms part of a controversial group of dairy bacteria [3], because antibiotic-resistant enterococci or virulence-factor genes possessing enterococci can cause human health disorders [4]. In general, based on the previous studies, the prevalent enterococcal species in raw milk have been found to be *Enterococcus faecium*, *E. faecalis* and *E. durans* [3]. Knowledge of these properties is required in order to reduce the pathogenic enterococcal strains from dairy production, and to eliminate the transfer of virulence-factor genes to other strains. It is also essential to find possible ways of eliminating them; the application of bacteriocins represents a promising approach, especially those with a broad antimicrobial spectrum [5–7]. The beneficial use of bacteriocins for these purposes has been confirmed several times under in vitro, in situ and in vivo studies [8,9]. As previously mentioned, the controversial character of enterococci lies in their beneficial as well as undesirable properties, especially in the beneficial production of proteinaceous antimicrobial substances—namely, enterocins [5]. In the past, the inhibition of *Staphylococcus aureus* SA1 in skim milk and yoghurt production was confirmed using enterocin CCM 4231 and its anti-listerial potential [8]. Moreover, enterocins showed benefit in food-producing animals. In broiler rabbits, they stimulated phagocytic activity, and increased the reparation ability of enterocytes [9]. An increase in body weight [10] was also noted, as was the reduction of *Eimeria* oocysts [9]. The most frequently studied enterocins are those produced by representatives of the species

E. faecium [5,11,12], but bacteriocins produced by strains of *E. durans* [13,14] have also been reported. Enterocins are known to have an inhibition spectrum against more or less related bacteria [15].

Gallidermin is a polypeptide containing the amino acid residues lanthionine, β-methyllanthionine or α,β-didehydroamino acids, which are able to build intramolecular thioether bridges. This antimicrobial bacteriocin was first found in the strain *Staphylococcus gallinarum* TU 3928. Members of this group of bacteriocins are called lantibiotics [16], to which nisin also belongs, and is commercially available. Nisin has garnered significant influence in the food industry since its discovery as an alternative biopreservative [17]. Nisin and gallidermin act predominantly against Gram-positive bacteria [16].

Milk has become a regular component of human consumption due to its originality. In Slovakia, in addition to raw cow milk and products made from it, ewe milk and its products are also very popular among consumers [18]. The consumption of raw goat milk and goat milk products has also increased because of their benefit to humans [19]. The specificity of goat milk lies, for example, in the fact that it has more abundant immunoglobulin content than human milk. Goat milk is also higher in calcium content compared with cow milk, contains trace elements and is full of vitamins. The content of some fatty acids in goat milk is also high [20].

Regarding the potential involved, this study focuses on the safety aspects of enterococci isolated from raw goat milk, with the intention of testing properties such as biofilm formation ability, virulence-factor genes, enzyme production, antibiotic profile, as well as hemolysis and/or deoxyribonuclease activity. To investigate the in vitro possibility of eliminating enterococci with pathogenic character, their susceptibility to the commercial lantibiotic bacteriocins nisin and gallidermin was tested, as well as their susceptibility to durancin ED26E/7 (identified in our laboratory), produced by the *Enterococcus durans* ED26E/7 strain from ewe milk lump cheese [14].

2. Materials and Methods

2.1. Sampling, Strains Isolation and Identification

Raw goat milk (a total of 53 samples) was collected from healthy goats in central and eastern regions of Slovakia. Altogether 283 goats were sampled. Samples (51) were taken from individual goats, and two pooled milk samples were taken from 132 goats. Treatment of the milk samples followed the standard microbiological method specified by the International Organization for Standardization (ISO 6887-1:2017). First, they were diluted in Ringer solution (1:9, Merck, Germany). Then dilutions were plated onto M-Enterococcus medium (Difco, Lawrence, KS, USA). Agar plates were cultivated at 37 °C for 48 h. Different pure colonies were picked up and submitted for identification. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Brucker Daltonics, Billerica, MD, USA) was applied to identify isolates. This identification method is based on bacterial protein "fingerprints" [21]. The preparation of bacterial cell lysates followed the producer's instructions, and results evaluation was based on the MALDI Biotyper 3.0 identification database (Bruker Daltonics). Allocation of isolates was performed according to their MALDI-TOF MS score evaluation based on highly probable species identification (score 2.300–3.000), secure genus identification/probable species identification (2.000–2.299) and probable genus identification (1.700–1.999). Reference strains included in the Bruker Daltonics database were used as positive controls. Strains evaluated with the same score value (identical) were excluded. Enterococci were inoculated on M-Enterococcus medium (Difco, USA), and were stored using the Microbank system (Pro-Lab Diagnostic, Richmond, BC, Canada).

2.2. Biofilm Formation

The ability of the identified strains to form biofilms was checked using Congo red agar [22]. Agar plates were inoculated with the tested enterococci, incubated at 37 °C overnight and checked at 48 and 72 h. Biofilm formation was assessed through the presence

of black colonies with dry crystalline consistency. Negative strains remained pink. The positive, standard biofilm-forming control strain was *Streptococcus equi* subsp. *zooepidemicus* CCM 7316 (kindly provided by Dr. Eva Styková, University of Veterinary Medicine and Pharmacy in Košice, Slovakia). Growth of the strains tested was compared with the growth of the positive control strain.

Moreover, the biofilm-forming ability of enterococci was assayed using the quantitative plate method according to Chaieb et al. [23] and Slížová et al. [24]. One colony of each strain picked up from Brain heart medium after cultivation for 24 h at 37 °C (Difco, NJ, USA) was inoculated into 5 mL Ringer solution (pH 7.0, 0.75% *w*/*v*). The suspension obtained corresponded to 1×10^8 cfu/mL. A 100 µL volume from that dilution was transferred into 10 mL of Brain heart infusion (BHI, Difco, USA). A 200 µL volume of dilution was transferred into microtiter plate wells (Greiner ELISA 12 Well Strips, 350 µL, flat bottom, Frickenhausen GmbH, Germany). The plate was incubated for 24 h at 37 °C, and the biofilm formed in the microtiter plate wells was washed twice with 200 µL of deionized water. It was then dried at 25 °C for 40 min. The attached bacteria were stained for 30 min at 25 °C with 200 µL of 0.1% (*w*/*v*) crystal violet in deionized water, and the dye solution was aspirated away. The wells in the microtiter plate were washed twice with 200 µL of deionized water. After water removal, the plate was dried for 30 min at laboratory temperature. The dye bound to the adherent biofilm was extracted with 200 µL of 95% ethanol. A 150 µL volume was transferred from each well into a new microplate well to measure absorbance in nm (A_{570}). An Apollo 11 Absorbance Microplate reader LB 913 (Apollo, Berthold Technologies, Oak Ridge, TN, USA) was used for this measurement. Testing was repeated in two independent runs with 12 replicates. Sterile BHI was used in each testing, serving as negative control. Biofilm-forming strain *Streptococcus equi* subsp. *zooepidemicus* CCM 7316 (kindly provided by Dr. Eva Styková, University of Veterinary Medicine and Pharmacy in Košice, Slovakia) served as positive control. Biofilm formation was evaluated as highly positive ($A_{570} \geq 1.0$), low-grade positive ($0.1 \leq A_{570} < 1.0$) or negative ($A_{570} < 0.1$), as per Chaieb et al. [23] and Slížová et al. [24].

2.3. Virulence-Factor Gene Detection

Genes for five virulence factors were tested. PCR amplification with the primers and conditions used followed the protocols according to Kubašová et al. [25] and Lauková et al. [26]. The genes tested were as follows: *gelE* (gelatinase), *esp* (enterococcal surface protein), *efaAfm* (*E. faecium* adhesin), *hylEfm* (hyaluronidase) and *agg* (aggregation substance). The PCR products were separated by means of agarose gel electrophoresis (1.2% *w*/*v*, Sigma-Aldrich, Saint Louis, USA) with 1 µL/mL content of ethidium bromide (Sigma-Aldrich) using 0.5× TAE buffer (Merck, Darmstadt, Germany). PCR fragments were visualized with UV light. The strains *E. faecalis* 9Tr1 (our strain, Lauková et al. [27]) and *E. faecium* P36 (Dr. Semedo-Lemsaddek, University in Lisbon, Portugal) were positive controls. The PCRs were carried out in 25 µL volume. Testing mixture consisted of 1× reaction buffer, 0.2 mmol/L of deoxynucleoside triphosphate, 3 mmol $MgCl_2$, 1 µmol/L of each primer, 1 U of Taq DNA polymerase, and 1.5 µL of DNA template. The cycling conditions followed the protocol as previously reported by Kubašová et al. [25] and Lauková et al. [26].

2.4. Enzyme Activity and Antibiotic Resistance Tests

Enzyme activity in the identified enterococci was tested with the API-ZYM system (BioMérioux, Marcy l'Etoile, France). Enzymes involved in the kit were as follows: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. An amount of 65 µL McFarland standard one inoculum was transferred into each well of the test plate. Incubation was performed for 4 h at 37 °C. After that time, the reagents Zym A and Zym B were added and enzyme activity was evaluated. Color intensity values from 0 to 5 and

their relevant values in nanomoles (nmoL) were assigned for each reaction according to the color chart supplied with the kit.

Following the EFSA rules for analyzing antibiograms, the CLSI [28] method was used together with antibiotic disks. Twelve antibiotics in disks (Oxoid, USA) were tested according to the suppliers' recommendation: clindamycin (Da 2 μg); novobiocin (Nov, 5 μg); ampicillin (Amp, 10 μg); erythromycin, azithromycin (Ery, Azm, 15 μg); streptomycin (S, 30 μg); chloramphenicol, rifampicin, vancomycin, tetracycline, kanamycin (C, R, Van, Tc, Kan, 30 μg) and gentamicin (Gn, 120 μg). Overnight culture (100 μL) of broth culture medium (BHI) was spread on Blooded BH agar (Difco, USA). Disks were placed on the agar surface. Then, plates were cultivated at 37 °C for 24 h. Inhibition zones were evaluated and expressed in millimeters according to the disk suppliers' recommendations.

2.5. Hemolysis and Deoxyribonuclease Activity

Hemolysis formation was checked by inoculating the cultures onto BH agar (Difco, USA) supplemented with 5% defibrinated sheep blood. Plates were incubated at 37 °C for 24 h. The presence/absence of clear zones around the bacterial colonies was read as α- and β-hemolysis, and negative strains featured γ-hemolysis [29].

To determine deoxyribonuclease activity, each strain was spread onto the surface of DNase agar (Oxoid, USA), and the agar plates were incubated for 24 h at 37 °C. The production of deoxyribonuclease can be evaluated on this medium. Colonies producing DNase hydrolyze the deoxyribonucleic acid (DNA) within the medium. After flooding and acidifying the agar with 1 N HCl, the DNA precipitated out; the agar became turbid with clear zones around the DNase-positive colonies.

2.6. Susceptibility of Enterococci to Nisin, Gallidermin and Durancin ED26E/7

Nisin was supplied in the product Nisaplin (Aplin and Barret, United Kingdom), in which activity of nisin is 1,000,000 IU. The dosage of nisin used in testing was prepared according to Lauková et al. [10]. Gallidermin, the pure substance supplied by Enzo Life Sci. Corporation USA, (MW2069.4), was used at a concentration of 0.5 mg/mL in 2 μL doses. This was decided based on the results of previous studies. Raw durancin ED26E/7 was prepared according to the previous report by Lauková et al. [14]. Enterococci were tested using the agar diffusion method [30]. Brain heart infusion supplemented with 1.5% agar (BHIA, Difco, Lawrence, KN, USA) was used for the bottom layer. For the overlay, 0.7% BHIA enriched with 200 μL of the indicator culture strain was used (A_{600} up to 1.0). Bacteriocin dilution (10 μL of nisin and durancin, 2 μL of gallidermin) in phosphate buffer (pH 6.5, ratio 1:1) was dropped on the surface of soft agar with each tested enterococcal strain, and the plates were incubated at 37 °C for 18 h. Clear inhibition zones around the doses of diluted bacteriocins were read. Inhibition activity was expressed in arbitrary units per milliliter (AU/mL), as the reciprocal of the highest two-fold dilution of bacteriocins demonstrating complete growth inhibition of the tested strain. Tests were performed twice. Positive control was the principal indicator, fecal *Enterococcus avium* EA5 strain (from piglet, isolated in our laboratory); inhibition activity reached up to 102,400 AU/mL.

3. Results

3.1. Strains Identification, Biofilm Formation and Virulence-Factor Detection

Among 53 samples of raw goat milk from two regions of Slovakia, three enterococcal species were detected: *E. faecium*, *E. hirae* and *E. mundtii*. The species *E. faecium* dominated with 11 strains (Table 1), followed by one strain of *E. hirae* and one strain of *E. mundtii*. Five out of eleven *E. faecium* strain species were evaluated, with highly probable species identification (2.300–3.000), five *E. faecium* strains as well as one strain of *E. hirae* were identified with evaluation corresponding to secure genus identification/probable species identification (2.000–2.299). *E. faecium* EF16/1 and *E. mundtii* EM 2/2 were identified with scores corresponding to probable genus identification (1.700–1.999).

Table 1. Enterococci detected in raw goat milk, with biofilm formation and virulence factor gene detection.

Strain	Score	Congo/72	Biofilm	*efaAfm* Gene
EF3/2	2.315	ng	0.081 (0.02)	nt
EF4/1	2.310	ng	0.090 (0.02)	nt
EF6/2	2.201	ng	0.092 (0.03)	+
EF10/2	2.006	ng	0.076 (0.07)	+
EF11/1	2.317	ng	0.089 (0.03)	ng
EF12/1	2.225	+	0.119 (0.35)	ng
EF14/2	2.257	+	0.108 (0.32)	ng
EF15/1	2.345	ng	0.089 (0.03)	+
EF16/1	1.894	+	0.113 (0.34)	+
EF18/1	2.352	ng	0.087 (0.03)	nt
EF23	2.200	+	0.101 (0.32)	ng
EH21	2.197	ng	0.90 (0.04)	+
EM2/2	1.939	ng	0.075 (0.01)	nt

EF: *Enterococcus faecium*; EH: *E. hirae*; EM: *E. mundtii*; ng: negative; nt: not tested; +: positive; Biofilm: absorbance at 570 nm (SD); Score: MALDI-TOF mass spectrometry evaluation score.

Using Congo red agar, most enterococci did not form biofilm (Table 1); however, four strains of *E. faecium* were found to have biofilm formation ability (EF12/1, EF14/2, EF16/1 and EF23). The strains *E. hirae* EH21 and *E. mundtii* EM2/2 were negative. The strains with biofilm formation ability not detected on Congo red agar were not biofilm-forming, as tested with the quantitative method ($A_{570} < 0.1$). Four biofilm-forming *E. faecium* strains were evaluated with low-grade positive biofilm formation ability (Table 1; $0.1 \leq A_{570} < 1.0$).

Enterococci were *gelE* gene, *hylEfm* gene, *esp* gene and *agg* gene absent. Gene *efaAfm* (*E. faecium* adhesin) was present in five *E. faecium* strains (EF6/2, EF10/2, EF15/1, EF16/1 and *E. hirae* EH21, Table 1), however this gene was not detected in biofilm-forming strains.

3.2. Enzyme Production, Antibiotic Susceptibility, Hemolysis and Deoxyribonuclease Activity

E. faecium strains EF3/2, EF4/1, EF6/2, EF11/1, EF16/1 and EF23 did not produce enzymes when tested. The strains EF 10/2, EF12/1 and EF14/2 showed high volumes of the enzyme β-galactosidase (30 nmoL, 20 nmoL), and in strains EF15/1 and EH21 we measured 40 nmoL of β-galactosidase. EH21 also showed 30 nmoL for β-glucosidase, and 20 nmoL was found in the case of strain EF15/1. The strains EF14/2, EF12/1 and EF10/2 produced β-glucosidase only at the 5 nmoL level. The other enzymes were not produced at all, or only in slight amounts (5–10 nmoL). Trypsin, α-chymotrypsin and naphtol-AS-BI-phosphohydrolase were not produced.

Enterococci from raw goat milk were mostly susceptible to antibiotics including ampicillin (inhibition zone size in range 11–18 mm), penicillin (14–20 mm), erythromycin (12–20 mm), vancomycin (15–20 mm), gentamicin (12–20 mm), chloramphenicol (12–25 mm), tetracycline (20–30 mm) and rifampicin (11–22 mm, except EF11/1 and EM2/2). However, they were resistant to kanamycin and clindamycin (Da). Most strains were also resistant to streptomycin (except EF18/1 and EM2/2). *E. faecium* EF6/2, EF14/2 and EF15/1 were resistant to Da and streptomycin (S 30 μg). EF10/2 was monoresistant (S 30 μg) as was EF16/1 (Azm, Table 2). *E. faecium* EF11/1 was resistant to four antibiotics (Da, Nv, S and R). The strains EF12/1 and EF18/1 were resistant to three antibiotics: Da, Nv (novobiocin 5 μg) and to S or Azm (EF18/1, Table 2). *E. mundtii* EM2/2 was found to have resistance against Da, S and R. *E. hirae* EH21 was susceptible to antibiotics; however, it possessed the *efaAfm* gene. EF16/1 was Azm resistant, biofilm-forming and possessed the *efaAfm* gene. Enterococci were hemolysis negative (γ-hemolysis) and deoxyribonuclease negative.

Table 2. Antibiotic profile of enterococci detected in raw goat milk.

Strains	6/2	10/2	11/1	12/1	4/2	15/1	16/1	18/1	EH21	EM2/2
Da (2 µg)	R	20 S	R	R	R	R	25 S	R	25 S	R
Nv (5 µg)	15 S	15 S	R	R	17 S	11 S	16 S	15 S	16 S	15 S
Azm (15 µg)	13 S	16 S	12 S	14 S	13 S	12 S	R	R	12 S	11 S
S (30 µg)	R	R	R	R	R	R	14 S	R	14 S	R

Da: clindamycin; Nv: novobiocin; Azm: azithromycin; S: streptomycin; S: susceptible, size of inhibition zone (mm); R: resistant, no inhibition zone. All strains were resistant to kanamycin. Strains were susceptible to vancomycin (15–20 mm), tetracycline (20–30 mm), chloramphenicol, rifampicin (30 µg), gentamicin (120 µg; 12–20 mm), erythromycin (15 µg), penicillin (10 IU; 14–20 mm), and ampicillin (10 µg; 11–18 mm).

3.3. Susceptibility of Enterococci to Treatment with Nisin, Gallidermin and Durancin ED26E/7

Enterococci were susceptible to treatment with bacteriocins. Pure-form nisin and gallidermin inhibited the growth of enterococci with high inhibition activity. Using nisin, inhibition activity ranged from 6400 AU/mL (against EF16/1) to 204,800 AU/mL (Table 3). Enterococci growth was inhibited using gallidermin, with activity from 200 to 204,800 AU/mL. Although durancin ED26E/7 was used only in raw substance, enterococci growth was still inhibited, but lower inhibition activity was measured (100–25,600 AU/mL, Table 3).

Table 3. Susceptibility of enterococci from raw goat milk to bacteriocins (AU/mL).

Strains	Nis	Gal	ED26E/7
EF6/2	204,800	204,800	25,600
EF10/2	51,200	204,800	6400
EF11/1	204,800	204,800	400
EF12/1	204,800	204,800	800
EF14/2	51,200	204,800	6400
EF15/1	12,800	3200	100
EF16/1	6400	6400	25,600
EF18/1	51,200	51,200	25,600
EF23	102,400	102,400	25,600
EH21	12,800	200	100

EF: *Enterococcus faecium*; EH: *E. hirae*. *E. mundtii* EM2/2, EF3/2 and EF4/2 were not tested. Nis: nisin; Gal: gallidermin; ED26E/7: durancin; AU/mL: arbitrary units per milliliter.

The biofilm-forming strain EF16/1 (Azm resistant, possessing the *efaAfm* gene) was inhibited with bacteriocins at a lower activity (AU/mL) than other strains (Table 3). Though still susceptible to all antibiotics used, *E. hirae* EH21, was the least susceptible to bacteriocins.

4. Discussion

In general, milk is an important nutritional component for populations worldwide, but it is also a useful matrix for a variety of bacteria [31]. Enterococci occur naturally in dairy products, as previously reported [19,32], with *E. faecium* and *E. faecalis* being predominant. Shirru et al. [33] also found a high prevalence of *E. faecium* strains in raw goat milk in Sardinia. Teržič-Vidojevič et al. [3] reported that the most prevalent enterococcal species in milk and cheeses were *E. faecium*, *E. faecalis* and *E. durans*. Usually, enterococci detected in milk are from the same or related cluster/group, which is reflected in the results of our study. Enterococcal species detected in the present study are from the same bacterial cluster/group (i.e., *E. faecium*, following gene similarity 16S rRNA analysis) [34]. Regarding raw goat milk or raw milk in general, enterococci can come from animal handling [35]. MALDI-TOF MS spectrometry has emerged as a tool for rapid microbial identification. This analytical technique is based on the ionization of chemical compounds into charged molecules, and the ratio of their mass to charge is measured [36]. Although it has been

shown that enterococci have little value as hygiene indicators in the industrial processing of foods, it is necessary to evaluate them in terms of their safety [34].

Hyaluronidase and gelatinase are hydrolytic enzymes which are involved in virulence factor levels in enterococci. Gelatinase gelE is an extracellular zinc metallo-endopeptidase. This gene is mostly secreted by *E. faecalis* [37]. The *gelE* gene is located on the chromosome. It is regulated in a cell-density-dependent manner, and hydrolyses gelatin, casein, hemoglobin and another bioactive peptides. Hyaluronidase is a degradative enzyme associated with tissue damage which acts on hyaluronic acid [38], and is encoded by the chromosomal *hyl* gene. The enterococci we tested were *gelE* and *hyl* gene absent. The presence of the *agg* gene (aggregation substance) increases the hydrophobicity of the enterococcal cell surface. It is a pheromone-inducible surface glycoprotein which mediates aggregate formation during conjugation [37]. This gene was also absent in enterococci isolated from raw goat milk. In this study, the *efaAfm* gene (*E. faecium* adhesin gene for adhesion to collagen) was detected in five strains of *E. faecium*. While it has not yet been conclusively shown to contribute to pathogenesis in animal experiments, it may nevertheless pose a safety risk [39].

Latassa et al. [38] reported that some Esp-negative *E. faecalis* strains were able to produce biofilms after receiving plasmid transfer of the *esp* gene. The enterococci we tested were *esp* gene absent, and only four strains were able to form biofilm (low-grade biofilm-forming ability). Mannu et al. [40] reported that the *esp* gene was absent in dairy isolates, which could be explained by the fact that Esp factor is thought to promote adhesion, colonization and evasion of the immune system. It is also thought to play some role in antibiotic resistance [6]. Enterococci can be intrinsically resistant towards antibiotics such as cephalosporins, β-lactams, sulfonamides and low levels of clindamycin and aminoglycosides [41]. As a result of their intrinsic resistance, antibiotic-resistant strains can frequently be found. Although phenotype testing for antibiotic resistance/susceptibility was performed in this study, enterococci from Slovak raw goat milk were mostly susceptible to antibiotics, except clindamycin (2 μg), kanamycin and streptomycin (30 μg).

Biofilm formation can also contribute to enterococcal pathogenicity and can act as persistent sources of contamination, leading to hygiene problems in food products [42]. However, in our tests, only low-grade ability to form biofilm was detected in four enterococcal strains. In addition, enterococci were hemolysis-negative (γ-hemolysis) and were DNase negative as well.

In our results from enterococci testing, it is particularly promising that the strains involved appear not to be pathogenic (mostly free of virulence-factor gene occurrence), and some of them even produce high amounts of β-galactosidase. This enzyme is used in the dairy industry for the production of lactose-free milk intended for lactose-intolerant humans [43]. Moreover, no production or only slight amounts (5 nmoL) of undesirable enzymes (e.g., naphtol-AS-BI-phosphohydrolase) were measured in our testing.

Although enterococci detected in Slovak raw goat milk appear not to be pathogenic, their potential elimination using bacteriocins is indicated. The strains were susceptible to bacteriocin treatment. The inhibition of enterococci through treatment with enterocins was also reported in our previous studies, for example, against fecal enterococci possessing virulence-factor genes [44]. Enterocin was also reported to inhibit *L. monocytogenes* and *S. aureus* in milk products [8], and the inhibitory effect of nisin in broiler rabbits was demonstrated after its application in drinking water [10].

5. Conclusions

The representatives of three enterococcal species in Slovak raw goat milk were found not to have pathogenic potential. The originality of this study, however, is in the fact that enterococci were susceptible to lantibiotic bacteriocin treatment, and to durancin ED26E/7 as well. Some of enterococci even produce high amounts of the β-galactosidase enzyme, which is used in the dairy industry for the production of lactose-free milk intended for lactose-intolerant people.

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Conflicts of Interest: The authors declare no conflict of interest.

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